United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-MTH-04		Page 1 of 5
Title: Detection of *Salmonella* in Fresh Produce by BAX® PCR		
Revision: 02	Replaces: 01	Effective: 01/01/06

1. Purpose

To provide a standard procedure for detection of *Salmonella* species in fresh produce using the BAX® system by all laboratories participating in the USDA/AMS Microbiological Data Program (MDP). The BAX® system is used here as a screening tool.

2. Scope

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

3. Principle

The BAX® PCR system is a DNA-based screening method developed by DuPont Qualicon for detecting bacterial pathogens in food and environmental samples. The sensitivity and the accuracy are a result of the use of polymerase chain reaction (PCR) to amplify DNA fragments specific for a given target organism. The amplification of specific DNA fragments is monitored using a dye that fluoresces upon intercalating with the double-stranded DNA of the amplified PCR product.

4. Outline of Procedures

5. References

5.1. BAX® System User Guide & Protocol Summary, DuPont Qualicon

5.2. SOP MDP-DATA-01 Record Keeping and Results Reporting

5.3. SOP MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction

5.4. SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce

5.5. SOP MDP-QA-03, Quality Assurance (QA) Controls

5.6. Evaluation of Enrichment Ability of Universal Pre-Enrichment Broth (UPB) for *Salmonella* ser. Typhimurium and *E. coli* O157:H7 from Produce Commodities. Final study report, Division of Consolidated Laboratory Services (DCLS), Department of General Services, Commonwealth of Virginia. October 2005.

6. Procedures

6.1. Equipment and Materials

6.1.1. BAX® System

6.1.2. BAX® PCR assay kit for *Salmonella* species

6.1.3. Additional materials needed to perform procedure as listed in BAX® System User Guide & Protocol Summary

Note: Use BAX® lysis buffer without protease.

6.2. List of Controls (Specific strains are listed in SOP MDP-QA-03)

6.2.1. DNA from Universal Preenrichment Broth (UPB) (Blank)

6.2.2. DNA from negative culture control from SOP MDP-LABOP-02

6.2.3. DNA from positive culture control from SOP MDP-LABOP-02

6.2.4. DNA from positive produce culture control from SOP MDP-LABOP-02

6.2.5. BAX® lysis buffer (without protease)

6.2.6. Carry all controls through this entire procedure, including any necessary cultural confirmation. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

6.3. BAX® Analysis

6.3.1. Use DNA extracted from the UPB preenriched cultures. Keep the DNA under refrigeration or in a cooling block until ready to use.

6.3.2. Transfer PCR tubes to the cooling block.

6.3.3. Hydrate BAX® reagent pellet by adding 50 µL of BAX® lysis buffer (without protease) to the PCR tubes.

6.3.4. Transfer a 5-µL aliquot of extracted DNA prepared from each of the UPB-preenriched samples and controls (from SOP MDP-LABOP-02) to the PCR tubes.

Note: To minimize contamination, keep samples and controls separate.

Note: Do not add DNA sample directly to the BAX® reagent pellet without prior addition of lysis buffer (without protease) alone or as a DNA:lysis buffer mix.

6.3.5. Proceed as directed by BAX® Users manual.

6.4. BAX®-Positive Samples

6.4.1. When a sample is positive or indeterminate via BAX®, proceed to attempt to isolate *Salmonella* as directed in SOP MDP-MTH-03A.

6.5. Reporting

6.5.1. A BAX®-positive result is considered a preliminary positive result.

6.5.2. Data shall be reported according to SOP MDP-DATA-01.

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

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Revision 02 January 2006 Monitoring Programs Office

- Added references to UPB
- Modified BAX® procedure to allow extracted DNA
- Screening procedure now performed on individual samples (rather than 3 pooled samples)
- All cultural procedures now included in SOP MDP-MTH-03A

Revision 01 January 2005 Monitoring Programs Office

- Removed references to outdated control strains
- Removed all duplicative instructions found in BAX® Users Manual
- Added provision to obtain 25 mL wash eluate for positive produce control